Chapter 19
Genomics of Biotic Interactions in the Triticeae

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Abstract In the area of Triticeae-pathogen interactions, highly parallel profiling of the transcriptome and proteome has provided entry points to examine host reaction to various pathogens and pests. In particular, the molecular mechanisms underlying gene-for-gene resistance and basal defense have been explored in the contrasting contexts of host vs. nonhost resistance and biotrophic vs. necrotrophic pathogenesis. Capitalizing on a rich history of genetics, molecular biology and plant pathology, recent studies in the Triticeae have provided new insights and characterized previously undescribed phenomena. The unique features of various pathosystems are increasingly leveraged by breakthroughs in genomic technologies, facilitating a community-wide approach to unifying themes of molecular plant-microbe interactions in the Triticeae.

19.1 Disease Epidemics and Current Threats

Plant diseases pose one of the greatest threats to agriculture in all corners of the world. In the Triticeae, major epidemics have occurred throughout history, affecting yield and quality of grain. Ergot of rye, which is caused by the ascomycete fungus, *Claviceps purpurea,* has possibly had more impact on the world than any other species of fungus. The earliest authenticated reports of its effects have been documented in Chinese writings around 1100 BC, and written accounts of wheat stem rust epidemics date back to ancient Greece and Rome.

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In modern agricultural systems, the genetic uniformity required for mechanized production renders crops vulnerable to severe losses. Stem, leaf, and stripe rusts have been found in most areas of the world (Kolmer 2005). For example, stem rust, caused by the obligate fungal biotroph *Puccinia graminis* f. sp. *tritici* (*Pgt*), has been a serious problem wherever wheat and barley are grown (Leonard and Szabo 2005; Roelfs 1985), including a new threat by a novel race of wheat stem rust (*Pgt* TTKS) from East Africa (Stokstad 2007; Wanyera et al. 2006). This race, commonly called Ug99, is virulent on many current wheat varieties as well as advanced lines in most breeding programs (Bonman et al. 2007; Jin et al. 2007; Jin and Singh 2006). *Pgt* TTKS infects barley as well as wheat, thus, comprehensive molecular and genetic approaches will be necessary to provide a detailed understanding of the interactions of stem rust with its host plants, as well as a comparative resource to delineate conserved and unique mechanisms of resistance to Triticeae pathogens.

Other important pathogens and pests, such as *Fusarium graminearum* (Fusarium head blight or scab) (Parry et al. 1995), *Blumeria graminis* (powdery mildew) (Jørgensen 1994), *Pyrenophera teres* (net blotch) (Serenius et al. 2005), *Barley yellow dwarf virus* (BYDV) (D'Arcy and Burnett 1995), and Hessian Fly (Liu et al. 2007b) also have been serious deterrents to Triticeae grain production worldwide.

19.1.1 Plant Defenses Employed in Response to Biotic Stress

Historically, cereal crops have laid the foundation for numerous classical genetic studies in host-pathogen biology, resulting in many model biological systems. This is in part due to the longstanding and continuous threat posed by rusts and mildews. As a result, "gene-for-gene" and "non-host" interactions with wheat and barley have been primary targets for intricate biological studies.

In gene-for-gene systems, plants are protected from disease by specific recognition of diverse effectors presented by invading pathogens. This recognition capacity is encoded by plant-resistance (*R*) genes, which initiate signaling cascades through direct or indirect recognition of a cognate pathogen avirulence (*Avr*) gene product (Axtell and Staskawicz 2003; Mackey et al. 2003). The most prevalent class of plant *R* genes encode putative intracellular receptors containing highly conserved motifs including an N-terminal coiled coil (CC) or Toll/Interleukin-1 receptor-like (TIR) domain, a nucleotide binding site (NBS), and C-terminal, leucine rich repeats (LRR; Jones and Takemoto 2004; Jones and Dangl 2006). Specific residues within the LRR domains are hypervariable and targets for diversifying selection, which is a major factor in the determination of disease resistance specificity (Ellis et al. 2000, 2007; Michelmore and Meyers 1998; Mondragon-Palomino et al. 2002; Noel et al. 1999). In addition, genetic diversity also exists in the requirements for additional components of disease response pathways (Muskett and Parker 2003; Shirasu and

Schulze-Lefert 2003). Pathogen contact and recognition results in the induction of multiple components of the resistance response, including pathogenesis-related (*PR*) genes, the elicitation of systemic acquired resistance (SAR), and hypersensitive cell death (HR) (Hammond-Kosack and Jones 1996). These *R* genes (and their associated responses) are exploited by plant breeders to offset deleterious yield loss due to pathogen infection. However, mutations can occur within dynamic pathogen populations that alter the capacity of the host's *R* genes to recognize the invading pathogen.

Nonhost resistance functions at the plant and pathogen species level (Ellis 2006; Mellersh and Heath 2003; Thordal-Christensen 2003), and thus, has the potential for targeted deployment in Triticeae crops to control host pathogens. Two models of nonhost resistance are currently espoused. The first postulates the absence of adapted pathogen effectors, thereby leading to a non-compromised Pathogen Associated Molecular Patterns (PAMP)-triggered defense response, which is durable in nature. This type of resistance is also known as basal resistance or innate immunity in host systems. The second model postulates that nonhosts harbor multiple *R* genes that collectively recognize several to many pathogen-derived effector proteins (Jones and Dangl 2006). Such redundancy is thought to confer durable resistance because it is unlikely to be defeated on multiple fronts simultaneously, precluding positive selection for potentially virulent pathogen effector alleles that arise. In order to test these hypotheses in *Triticeae*, experimental approaches that overcome the obstacle of genetic separation of host- versus non-host plants, which usually belong to different species, will be essential.

19.1.2 Integrative Genomics Holds the Keys to Durable Resistance

Since the dawn of agriculture, the rate of crop improvement has been accelerating, making it possible for several billion people to eat without being involved in food production. In turn, trends toward globalization are heightening the epidemiological challenges facing agronomists and growers alike. Whereas *R*-genes could previously be used for crop protection until new variants arose in pathogen populations, migration of pre-existing virulent pathovars may become the more common source of defeat. This scenario emphasizes the general need to identify and utilize broader spectrum basal defense mechanisms that will confer durable resistance. A challenge herein is that these mechanisms often have only incremental effects on resistance when studied individually, making them much more difficult to identify experimentally. In order to create the germplasm that will thrive in these modern times, accelerated discovery of genic and biochemical targets and rapid application to crop improvement must be achieved. In this regard, transferring knowledge learned from model systems to agricultural applications lies in systems biology approaches that integrate complementary genomics experiments.

19.2 The Toolbox for Investigating Biotic Interactions

Genomic approaches commonly in use for investigating biotic interactions in the Triticeae generally fall into one of three categories: profiling of molecules to discover differential responses to treatments, integration of phenotypic, genetic and physical data to demonstrate consequences of an allelic difference, and systematically testing hypothetical functions of genes via stable and transient manipulations. Most investigations use a combination of these approaches, as they typically have complementary strengths. We review these in the context of the challenges posed and opportunities availed by interactions between pathogens and their Triticeae hosts.

19.2.1 Molecule Profiling Approaches

Molecule profiling approaches characterize biochemical and physiological differences that arise in response to treatment, genotype or both. Substantive variation manifests at all levels, including but not limited to differences in chromatin acetylation, DNA methylation, promoter function, splice variation, posttranscriptional antagonism, protein production and modification, sub-cellular targeting and finally, interaction with other required molecules. In both biological and technical terms, characterization of the transcriptome is by far the most tractable; it is the essential intermediate between the genome and the proteome and is readily characterized in its reverse transcribed state. Not surprisingly then, at least a plurality of current discoveries utilize transcriptomic experiments as entry points.

Profiling of mRNA in the Triticeae has been greatly facillitated by the development of Affymetrix GeneChips for both barley (Close et al. 2004) and wheat, facilitating acquisition of high quality data using platforms that gain utility as more experiments leverage them (Wise et al. 2007a). The Barley1 GeneChip assays mRNA levels for 21,439 unique genes and has been utilized extensively to analyze time-course expression profiles of hosts challenged by several pathogens (Wise et al. 2007b). While numerous discoveries have been made using this and other microarray platforms (see below), a limitation is that the expression levels for perhaps half of all barley genes are not queried. Although, expressed sequence tag libraries derived from infected materials were well represented in the unigene set to design the Barley1 GeneChip (Close et al. 2004), the application of next-generation deep sequencing technologies (e.g., 454 Life Science, Illumina/Solexa and ABISOLiD) to produce hundreds of millions of mRNA sequence tags will make more comprehensive microarrays possible within several years (Kling 2005; Margulies et al. 2005).

The Wheat GeneChip assays 55,052 transcripts, or about one third of all wheat genes, and is gaining momentum in the field of host-pathogen interactions (Coram et al. 2008; Liu et al. 2007b). However, proteomic profiling

by 2D electrophoresis has been more widely used thus far (Pós et al. 2005; Rampitsch et al. 2006; Wang et al. 2005; Zhou et al. 2005). This approach has a comprehensive potential in theory, but is limited by both sensitivity and electrophoretic resolution. It has the advantage of assaying gene products, rather than molecular intermediates, and can detect differences in phosphorylation states that can govern functionality if phosphoproteomic methods are employed (Rampitsch et al. 2006). A particular advantage of proteomic profiling in host-pathogen interaction studies is that the pathogen's proteome is also profiled. These studies will become increasingly valuable as plant and pathogen genomes are sequenced.

MicroRNAs have been shown to be an integral part of the defense response in Arabidopsis and loblolly pine, but characterization of their role in biotic stress response in Triticeae species has not yet been investigated (Lu et al. 2007; Navarro et al. 2006). However, recent microRNA profiling in wheat resulted in the identification of 23 new microRNA families, demonstrating the discovery power of deep sequencing approaches (Yao et al. 2007).

Success with profiling methods often depends on maximization of the signal-to-noise ratio. Single-cell transcriptomes have been profiled in barley to see what's different between a challenged and an unchallenged cell (Gjetting et al. 2004). In wheat, the intercellular secretome has been analyzed by proteomic methods to determine how defense responses at the point of contact are deployed (Pós et al. 2005). Even the method of RNA isolation can be optimized to better reflect the changing proteome in a transcriptome profiling experiment by hybridizing only cRNA associated with polysomes during translation (Skadsen and Jing 2008). Utilization of such biochemical properties will be required to identify the many types of molecular mechanisms underlying variation in defense response.

19.2.2 Integration of Phenotypic, Genetic and Physical-Map Data

Integration of phenotypic, genetic and physical-map associated sequence data to demonstrate consequences of allelic differences takes on divergent forms depending on whether the allelic differences in question are qualitative or quantitative. Map-based cloning (aka: positional cloning) integrates these data types to prove that a nucleotide sequence difference (typically qualitative) has a phenotypic consequence (see Chapter 12). This approach has been successfully used in the cloning of *R* genes responsible for triggering host resistance (Brueggeman et al. 2002; Halterman et al. 2001, 2003; Halterman and Wise 2004; Huang et al. 2003; Shen et al. 2003; Srichumpa et al. 2005; Wei et al. 1999, 2002; Yahiaoui et al. 2004).

Transcript-based cloning of genes using DNA microarrays has been used to discover important host-pathogen interactions as well. Expression values that are drastically lower in the mutant as compared to wild-type, such as those caused by deletion mutations, are the easiest to characterize (Mitra et al. 2004;

Zakhrabekova et al. 2002). This approach was used in barley to identify candidate genes encoding *Rpr1*, a gene required for *Rpg1*-mediated resistance to stem rust. A large deletion was identified via Barley1 GeneChip profiling of *rpr1* mutants derived from fast-neutron bombardment (Zhang et al. 2006). Three genes within the deletion cosegregated with the *rpr1*-mediated susceptible phenotype, narrowing the candidate gene list to facilitate characterization of *Rpg1*-specified resistance to stem rust.

Fast neutron mutants are well-suited for transcript-based cloning because the genetic lesions typically affect multiple genes and thus increase the likelihood of detecting expression knockouts (Alonso and Ecker 2006). However, since the deletions can vary in size from a single nucleotide to 30 kb, there is a potential of few to many genes that may need to be tested for co-segregation, and subsequent functional analysis performed.

When natural allelic differences are of interest, quantitative trait locus (QTL) mapping is typically the entry point. QTL mapping finds statistical associations between genotypes and phenotypes, allowing regions of the genome harboring allelic differences that cause variation in the phenotype to be identified (Mackay 2001). QTL mapping of infection type data has been widely used by plant pathologists to characterize the inheritance architecture of disease resistance and to identify chromosomal regions harboring regulators of defense (Horsley et al. 2005; Jiang et al. 2007; Leonova et al. 2007), but has not led to the cloning of genes other than those with qualitative effects. This is largely due to limitations in genetic resolution, which thwart efforts to singularly associate these causal differences with DNA sequence polymorphisms. Transcriptomic experiments tend to have a complementary deficiency; many DNA sequences are associated with differential response, but cause and effect relationships are not revealed. One of the most powerful approaches to elucidate consequences of natural allelic variation is to employ genetical genomics, a fusion of QTL mapping and high throughput genomic data collection techniques (Jansen and Nap 2001).

Expression QTL (eQTL) mapping is the most common genetical genomics approach. Transcript abundance of each single gene is treated as a quantitative trait and its regulation is genetically interrogated using QTL mapping approaches (Chen and Kendziorski 2007; Rockman and Kruglyak 2006). Since variation in gene expression has been shown to be a primary basis for the dynamic responses observed in plant-pathogen interactions, genetic interrogation of global gene expression during pathogen invasion is an appropriate way to identify and characterize defense gene networks (Hansen et al. 2008). By profiling gene expression in each member of a segregating population, it is possible to use linkage and network analyses to identify key regulators of gene expression for a particular condition (Jansen and Nap 2001; Rockman and Kruglyak 2006; Williams et al. 2007). eQTL mapping has been used in wheat to identify loci that regulate seed development (Jordan et al. 2007), and in barley to build gene networks associated with *R*-gene mediated and basal defense mechanisms involved in stem rust resistance (M. Moscou, N. Lauter, J. Rodriguez, G. Fuerst, B. Steffenson, Y. Jin, L. Szabo and R. Wise, unpublished results).

19.2.3 High-Throughput Functional Analysis

High-throughput functional capabilities for testing candidate genes are rapidly expanding. Functional validation of the candidate genes can be accomplished by genetic mutation, overexpression, or gene silencing (Caldwell et al. 2004; Douchkov et al. 2005; Hein et al. 2005; Scofield et al. 2005; Makandar et al. 2006). A number of resources needed for reverse genetic and functional analysis of candidate genes are now available for both plants and pathogens. Improvements in stable transformation (Fang et al. 2002; Hensel et al. 2008; Jacobsen et al. 2006; Janakiraman et al. 2002; Jones 2005) have benefited host-pathogen interaction studies just as they have other disciplines, but still remain a limitation. TILLING (Targeting Induced Local Lesions in Genomes, see Chapter 13) is also increasingly viable, but is often constrained by pathologically inappropriate genetic backgrounds, requiring introgression prior to functional characterization of alleles.

The most promising functional analysis breakthroughs for molecular plant pathologists are improvements in RNA interference (RNAi) techniques that alter gene expression in local cells or tissues that are the targets of pathogen invasion. Due to its sequence-homology dependent mode of action, double-stranded RNA interference (dsRNAi) is unique in its potential to overcome the problem of genetic redundancy, which is of critical importance for the polyploid genomes of Triticeae species (Chuang and Meyerowitz 2000; Schweizer et al. 2000; Tavernarakis et al. 2000).

Ten years ago, pioneering work by Bushnell and colleagues demonstrated the principle usefulness of a single-cell transient expression assay for powdery-mildew attacked barley (Nelson and Bushnell 1997). This assay was initially based on bombarded coleoptile tissue and subsequently developed for use in detached barley leaves (Nielsen et al. 1999; Schweizer et al. 1999b; Schweizer et al. 2000). This was possible because both the microprojectile-mediated transformation as well as powdery-mildew attack and potential development are cell-autonomous events taking place in single epidermal cells. The reliability of results obtained by using the transient assay has been verified by its ability to phenocopy allele introgression or gene mutation in barley and wheat, and by transgenic plants stably expressing genes of interest in leaf epidermis (Altpeter et al. 2005; Schultheiss et al. 2005; Schweizer et al. 2000).

The single-cell assay was further developed for transient-induced-gene-silencing (TIGS) by the expression of RNAi hairpin constructs (Douchkov et al. 2005). However, a limitation of the TIGS assay for barley-powdery mildew system has been the laborious phenotyping required to identify transformed cells and assign an infection severity rating based on haustorial features. Throughput is now significantly enhanced through use of microscope robotics and automated image analysis (Fig. 19.1) (Douchkov et al. 2005). Improvements in construct preparation using the Gateway™ technology have also accelerated this technique, such that 300 genes per person per

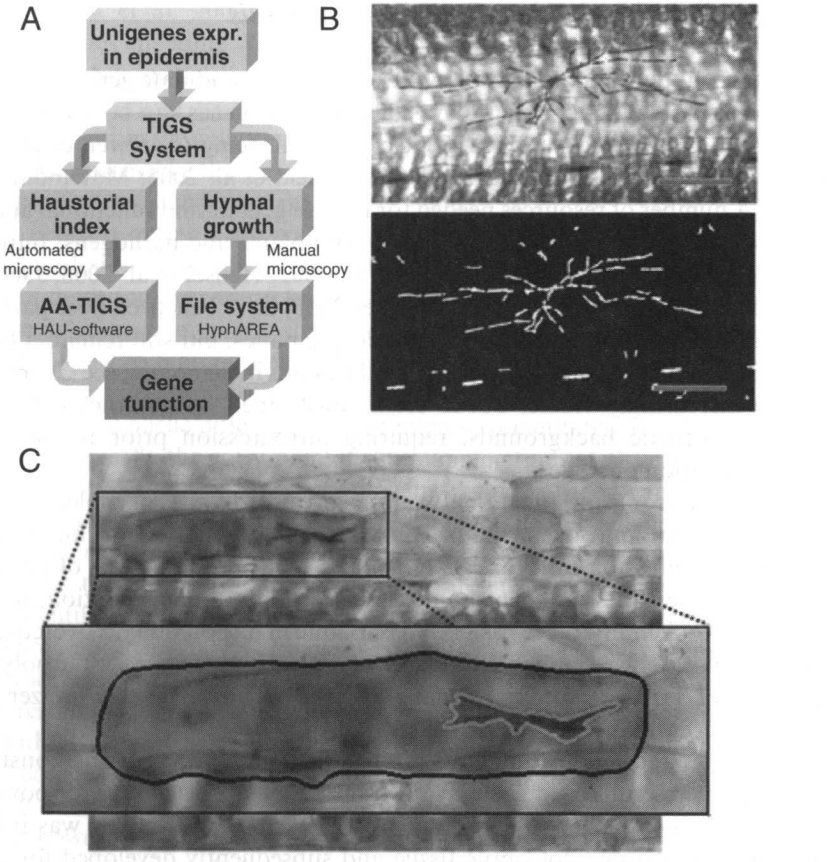

Fig. 19.1 High-throughput Transient-Induced-Gene-Silencing (TIGS) pipeline in barley. (**A**) Summary of approaches and tools available for quantitative phenomics in the barley-powdery mildew interaction: 1) Haustorial index is defined as the number of detected haustoria, divided by the number of observed GUS-positive, epidermal cells per bombardment. 2) Hyphal growth designates the increase of pixel numbers attributed over time to growing fungal colonies, as determined by the HyphAREA software tool. 3) File system designates the image files stored locally on a PC of data input into HyphAREA. 4) AA-TIGS and HAU-software is a Oracle database of microscopic images (input for the haustoria-recognition software) and quantitative microscopic data (output of the haustoria-recognition software) respectively. (**B**) Automated pattern recognition for quantitative assessment of fungal growth on the leaf surface. Upper panel, original image of a growing pustule, stained with Coomassie blue. Lower panel, final segmentation of hyphae for pixel quantification. Scale bar = 100 μm. (**C**) Example of transformed epidermal cell expressing the *GUS* reporter gene prior to and after automated segmentation of cell (black bordering line) and haustorium (red bordering line). The figure was derived from a figure in Schweizer et al. (2006) Hochdurchsatz-Phänomanalyse in Getreide. GenomXPress 2/06: 16–19. GenomXPress is the non-indexed journal of the German genomics programs including GABI (*See* Color Insert)

month can be analyzed with the automated single-cell transient assay if two robotic microscopes are used. Since the initial reports, a considerable amount of functional information concerning defense-related barley and wheat genes has become available (Summarized in Table 19.1). In addition to the single-cell haustorium assay, an automated quantitative assessment of hyphal growth rate of powdery mildew has been developed, which can be used to study transgene effects manifested later in pathogenesis, permitting analysis of the slow mildewing phenomenon (Göllner et al. 2008; Seiffert and Schweizer 2005).

Table 19.1 Genes silenced or overexpressed using biolistic and virus-induced transient assays in Triticeae-fungal interactions (Updated from Panstruga 2004)

Category (Gene name)[a]	Description	OEx	RNAi[b]	Reference
Defense				
TaPrx103 (WIR3)	Peroxidase	X		(Schweizer et al. 1999b)
TaWIR2	Thaumatin	X		(Schweizer et al. 1999b)
TaGluc	β-1,3 glucanase	X		(Schweizer et al. 1999b)
TaCH126	Chitanase	X		(Schweizer et al. 1999b)
TaGOX	Glucose oxidase	X		(Schweizer et al. 1999b)
HvPrx75; -85	Peroxidase	X		(Kristensen et al. 2001)
TaOXOX	Oxalate oxidase	X		(Schweizer et al. 1999a)
TaGLP4	Germin-like	X		(Schweizer et al. 1999a)
HvGER3; -4; -5	Germin-like proteins	X	TIGS	(Zimmermann et al. 2006)
HvADF3	Actin-depol. factor	X		(Miklis et al. 2007)
Resistance signaling				
HvMla alleles	CC-NBS-LRR protein	X	TIGS/ VIGS	(Halterman et al. 2001; Halterman et al. 2003; Halterman and Wise 2004; Halterman and Wise 2006; Shen et al. 2003; Shen et al. 2007; Zhou et al. 2001)
TaPm3 alleles	CC-NBS-LRR protein	X		(Srichumpa et al. 2005; Yahiaoui et al. 2006; Yahiaoui et al. 2004)
HvSgt1; HvRar1	SCF complex		TIGS/ VIGS	(Azevedo et al. 2002; Hein et al. 2005)
TaSgt1; TaRar1			VIGS	(Scofield et al. 2005)
HvRacB	Small GTP-binding protein	X	TIGS	(Schultheiss et al. 2003; Schultheiss et al. 2002)
HvG (alpha)	G protein subunit	X	TIGS	(Kim et al. 2002)

Table 19.1 (continued)

Category (Gene name)[a]	Description	OEx	RNAi[b]	Reference
Cell death				
HvMlo	7-Transmembrane protein	X	TIGS	(Kim et al. 2002; Schweizer et al. 2000; Shirasu et al. 1999a; Shirasu et al. 1999b)
TaMlo	7-Transmembrane protein	X		(Elliott et al. 2002)
HvBI-1	BAX inhibitor-like	X	TIGS	(Huckelhoven et al. 2003)
HvCam-HvMlo	Mlo-Calmodulin complex	X		(Kim et al. 2002)
HvRBOH	NADPH oxidase		TIGS	(Trujillo et al. 2006)
Transcription factor				
HvNAC6	NAC transcription factor	X	TIGS	(Jensen et al. 2007)
Avr_R_WRKY complex	Complex of *Bgh* AVR proteins with corresponding R proteins and WRKY transcription factors	X	VIGS	(Shen et al. 2007)
Vesicle traffic				
HvSNAP34	Syntaxin-interacting protein	X	TIGS	(Collins et al. 2003; Douchkov et al. 2005)
HvUbi	Ubiquitin	X	TIGS	(Dong et al. 2006)

[a]The prefix "*Ta*" or "*Hv*" specifies wheat or barley, respectively.
[b]TIGS designates Transient Induced Gene Silencing. VIGS designates Virus Induced Gene Silencing.

More recently, virus induced gene silencing (VIGS) has emerged as a powerful reverse genetics tool for the functional analysis of gene candidates in both model and crop plant species. In monocots, *Brome mosaic virus* (BMV) has been utilized for functional genomics studies in rice and maize (Ding et al. 2006), whereas, *Barley stripe mosaic virus* (BSMV) has been developed for use in barley and wheat (Hein et al. 2005; Holzberg et al. 2002; Lacomme et al. 2003; Scofield et al. 2005). BSMV-VIGS has been used to silence genes involved in barley (Hein et al. 2005) and wheat (Zhou et al. 2007) defense by demonstrating resistance-breaking phenotypes in the host plants against powdery mildew and rust pathogens, respectively. As with bombardment, BSMV has also been developed as a novel vector for systemic transient overexpression expression, providing additional functional testing capabilities (Tai and Bragg 2007).

19.3 Triticeae-Fungal "Host" Interactions

Of the current threats, rusts and mildews are biotrophs, and Fusarium is a hemibiotroph and a necrotroph. Host-pathogen interaction studies have focused on biotrophs because of their significance, and also because the obligate nature of the interaction allows good control against accidental escape. As a result, several of the main themes in molecular plant-microbe interactions have been addressed using Triticeae-biotroph interactions. The fact that rice and maize are nonhosts for some of these diseases has allowed the further exploitation of these systems for the mining of biotic factors in disease defense.

Commonalities in host transcriptomic responses across diverse interactions between barley and the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (*Bgh*), demonstrate that regardless of plant genotype, perception of general elicitors from pathogens leads to the induction of basal-defense responses (Caldo et al. 2004). These generalized defense mechanisms are thought to be targets for suppression by pathogen effector molecules deployed to establish infection (Alfano and Collmer 2004; Caldo et al. 2004; Espinosa and Alfano 2004). By extension, differences in the host gene expression patterns triggered by elicitors may provide insight into how pathogens respond and counter-attack early plant defense mechanisms (Caldo et al. 2006).

Transcriptomic investigations of *Mla*-mediated barley-powdery mildew interactions have also established a regulatory link between basal defense and *R*-gene mediated resistance, addressing one of the major questions outlined above (Caldo et al. 2004, 2006). A highly co-regulated cluster of >160 basal-defense related genes is significantly up-regulated in both incompatible and compatible interactions, coinciding with germination of *Bgh* conidia and formation of appressoria. Later, during establishment of the perihaustorial interface between penetrating *Bgh* and host epidermal cells, divergent expression of these transcripts occurs, where compatible interactions lead to lower expression compared to paired incompatible interactions. Many of these genes are associated with the basal defense response, which can be induced in a nonspecific manner by PAMPs (pathogen-associatedmolecular-patterns) or MAMPs (microbe-associated-molecular-patterns) (Bent and Mackey 2007; Chisholm et al. 2006). The regulatory link between basal defense and *R*-gene mediated resistance has been confirmed by numerous subsequent studies (Kim et al. 2005; Navarro et al. 2004; Shen et al. 2007; Wise et al. 2007b). Significantly, the nuclear localization of MLA interactors indicates that two of these basal resistance regulators are a pair of transcription factors, *Hv*WRKY1 and *Hv*WRKY2 (Shen et al. 2007). Homologues of *Hv*WRKY1/2 in Arabidopsis, *At*WRKY18/40, appear to be involved in a feedback repression system regulating deployment of basal defense (Shen et al. 2007).

Many outcomes are possible when thousands of powdery mildew conidia land on the same leaf. For example, one *Bgh* conidiospore may succeed during

the infection process and form a functional haustorium, whereas another may not. In order to investigate single-cell penetration attempts, Lyngkjaer and colleagues developed a system to micro-manipulate single barley epidermal cells (Gjetting et al. 2004) and examined differences in transcript accumulation following successful or unsuccessful penetration attempts. mRNA was captured on magnetic beads, purified and amplified for hybridization to the IPK (Gatersleben) barley PGRC1 10 k cDNA array (Sreenivasulu et al. 2001). Notably, up-regulation of sucrose synthase was exclusive to infected cells (Gjetting et al. 2007). In addition, two hexose transporters were up-regulated in both resistant and susceptible cells as well as genes associated with sucrose transport. Analogous findings have been observed in inoculated as compared to non-inoculated control samples from seedling leaves by Caldo and associates (2006); accession BB2 at http://plexdb.org/.

Bgh infects epidermal cells of barley, but also induces local acquired resistance in wheat, a nonhost (Bruggmann et al. 2005; Eichmann et al. 2006). To determine the extent to which epidermal penetration affects the disease response in mesophyll cells, incompatible responses of wheat were assayed in RNA samples enriched for epidermal or mesodermal leaf tissue. As expected, a large number of defense proteins were induced, but surprisingly, most had a greater fold change in the mesophyll than in the epidermis, indicating that local signaling occurs (Bruggmann et al. 2005).

Boddu and colleagues (Boddu et al. 2006), performed an extensive time-course study of barley cv. Morex spikes infected with *F. graminearum* isolate Butte 86 as compared to mock water control sampled at 24, 48, 72, 96, and 144 hai (Boddu et al. 2006; Accession no. BB9 at http://plexdb.org/). Interestingly, a large number of overlapping and conserved gene expression patterns were observed among these experiments and diverse host-pathogen interactions involving barley-*Bgh* (Caldo et al. 2004, 2006) and Arabidopsis response to its host powdery mildew, *Erysiphe orontii* (Wise et al. 2007b; Pathogenomics Integrated Microarray Database System: IMDS, http://ausubellab.mgh.harvard.edu/imds/, data downloaded November, 2006).

Trichothecene mycotoxin accumulation is associated with the shift from biotrophic to necrotrophic infection stages (Bushnell et al. 2003) and reduced virulence is correlated with low deoxynivalenol (DON) concentration in wheat (Mesterhazy et al. 1999). To understand the role of *F. graminearum* trichothecene mycotoxins in the barley host response, Boddu and associates (Boddu et al. 2007) investigated spikes of barley cv. Morex infected with trichothecene-producing wildtype strain (Z3639) and loss-of-function *tri5* trichothecene nonproducing mutant (GZT40). RNA was extracted at 48 and 96 hai for expression profiling (Boddu et al. 2007; Accession no. BB52 at http://plexdb.org/). This experiment revealed a unique set of 63 trichothecene-induced transcripts, in addition to accumulation of basal defense transcript observed by inoculation with the wild type strain of *F. graminearum*. These trichothecene-induced transcripts appear to control multiple cellular responses, including trichothecene detoxification and transport, ubiquitination, programmed

cell death, transcription, and secondary metabolism. Boddu et al. (2007) propose that trichothecene induces at least two classes of genes: (1) those involved in trichothecene detoxification, including UDPglucosyltransferases, ABC and MATE transporters, and (2) those encoding ubiquitination- and programmed-cell-death-related genes, including AAA-family ATPases, U-box domain protein, NF-X1 zinc finger protein, F-box protein, and a pirin protein. Thus, for the necrotrophic phase of *F. graminearum*, cell-death associated host gene expression likely enhances disease progression.

19.4 Triticeae-Fungal "Nonhost" Interactions

In the *Triticeae*, several wide interspecific introgressions of chromosome fragments confer strong and durable resistance in both wheat and barley (Mago et al. 2005; Oliver et al. 2005; Rubiales et al. 2001; Ruge et al. 2003; Tang et al. 1997). The basis for resistance in these cases is not clear; multiple *R* genes or major-effect "nonhost" defense genes are just two of the possibilities.

Progress has recently been obtained in barley attacked by inappropriate *formae speciales* (f. sp.) and species of the powdery mildew fungus *Blumeria graminis* and the rust fungus *Puccinia* sp., respectively. These obligate biotrophic rust and powdery mildew pathogens usually exhibit a high degree of host specificity, which means that they only infect one or a few closely-related hosts such as barley (*Hordeum* sp.) or wheat species (*Triticum* sp.). Therefore, a clear distinction of host *versus* nonhost interactions is possible allowing comparative or functional approaches. The focus on these pathosystems, together with the employment of multiple successive crosses, mutagenesis and gene-silencing approaches in barley opened up new possibilities to discover genes involved in nonhost resistance and to unravel the underlying mechanisms (Schweizer 2007).

In spring barley, the *mlo*-resistance gene has been widely used because it conferred race-nonspecific and durable resistance against *Bgh* for over 20 years. This provokes the question: Do *mlo*-mediated and nonhost resistance share common pathways that, in the case of *mlo*-mediated resistance, appear to result in strong basal resistance relieved from negative control by the MLO protein? There is ample evidence from physiological and transcript-profiling experiments in the barley-*Bgh* pathosystem that negative control of defense is indeed triggered or enhanced by successful fungal spores with MLO being one of the potential fungal effector (suppressor) targets (Lyngkjaer et al. 1997). The *PEN1* (for *penetration1*) and *Ror2* (for *required for mlo resistance 2*) genes have been found to encode functionally homologous tSNARE proteins in *Arabidopsis* and barley, respectively, that are predicted to mediate Golgi-vesicle fusion with target membranes (Collins et al. 2003). Although *Ror2* was originally identified in a mutant screen for breakdown of *mlo*-mediated host resistance to *Bgh*, it was recently found also to contribute to nonhost resistance, together with *Ror1*, another gene of unknown function required for *mlo*-mediated resistance

(Trujillo et al. 2004). The HvSNAP34 protein, which is similar to the yeast tSNARE protein SNAP25 (for synaptosome-associated protein of 25 kd) and predicted to interact with ROR2 in a ternary SNARE complex at target membranes, was found to be required for *mlo*-mediated host resistance against *Bgh* as well as for nonhost resistance to *B. graminis* f. sp. *tritici (Bgt)* (Douchkov et al. 2005). These results were obtained by transient-induced gene silencing (TIGS) in bombarded epidermal cells. The same dual functionality may be true for the *BI-1* gene encoding Bax-inhibitor 1, a putative negative regulator of cellular defense, and for the *ADF3* gene encoding the actin-depolymerizing factor 3 of barley because transient overexpression caused breakdown of nonhost- as well as *mlo*-mediated resistance (Eichmann et al. 2004; Huckelhoven et al. 2003; Miklis et al. 2007). Finally, *Mlo* overexpressing epidermal cells became susceptible to this inappropriate mildew suggesting that the cells use the same defense pathway that is under negative control by the MLO protein for basal host- and nonhost resistance (Elliott et al. 2002). In summary, mutant and reverse-genetic approaches in barley have produced results that suggest a functional overlap of *mlo*-mediated with nonhost resistance against powdery mildew.

In the interaction of barley with rust fungi, a better understanding of the genetic basis of nonhost resistance was achieved recently by accumulating alleles for nonhost susceptibility in a series of crosses, which resulted in two barley lines with essentially full susceptibility to inappropriate rust fungi (Atienza et al. 2004). This was possible because one of the parents of the initial cross, line L94, showed some marginal nonhost susceptibility to *P. triticina*. As a consequence, transgressive segregation of nonhost susceptibility was observed and exploited for consecutive crosses. Segregation analysis of progeny resulting from a series of crosses between "normal" nonhost resistant parents and one of the new, nonhost susceptible lines yielded a first insight into the genetic basis of the (non)host status of barley and revealed rather complex sets of mostly non-overlapping loci depending on the combination of parents (Jafary et al. 2006). Interestingly, map positions of a number of defense-related candidate genes in the progeny from these crosses were in significant association with nonhost-resistance QTLs. Therefore, it is tempting to speculate that not only in the interaction of barley with powdery mildew but also in barley-rust interactions, nonhost resistance is brought about by non-suppressed basal resistance that depends on a combination of defense-related alleles, which may vary from genotype to genotype and which reflects a considerable inherent degree of functional redundancy.

In contrast to barley, wheat might rather employ major *R*-genes with very durable effects for nonhost resistance to powdery mildews, as derived from inter-*formae-specialis* crosses of powdery mildew fungi. It was found that often one or a few segregating genes of the fungus were deciding upon host range, together with one or a few genes in the nonhost that co-segregated with known *R*-genes against *Bgt* such as *Pm10* (Matsumura and Tosa 1995; Tosa 1989). However, it remains a riddle why two as closely-related species as barley and wheat should differ in basal mechanisms of nonhost resistance against powdery

mildew fungi. Wheat would not be the only system in which *R*-gene mediated nonhost resistance has been observed because similar findings were reported for soybean (*Glycine max* L.) and *Arabidopsis* (Kobayashi et al. 1989; Staal et al. 2006). Since cultivated wheat has homoeologous A, B, and D genomes, an alternative explanation may be that knockout mutations specifying nonhost phenotypes have not yet been identified. In summary, the current data rather favor a model of nonhost resistance in barley that depends on a noncompromised, PAMP-triggered basal defense. This model is based on the assumption that fungal effectors (defense suppressors) released by inappropriate pathogens are largely ineffective against nonhosts. Future efforts on map-based cloning of nonhost QTLs and TILLING of candidate genes will provide a clearer picture of the genetic and molecular basis of nonhost resistance in Triticeae crops.

19.5 Triticeae Interactions with Insects, Viruses, Worms and Bacteria

Triticeae crops suffer significant damages from viruses, nematodes, insects and bacterial pathogens as well. *Barley yellow dwarf virus* (BYDV) and *Wheat streak mosaic virus* (WSMV) are potyviruses that cause significant economic losses, prompting major efforts to breed disease resistant varieties (Hakizimana et al. 2004; Sip et al. 2006). As is true for bacterial diseases, Triticeae species are not the best model hosts for genomics research on these interactions, so little is known about molecular genetic basis for the resistance mechanisms that breeders are using to protect small grain crops. The case is similar for Triticeae interaction with cereal cyst nematodes, as well as other parasitic worms that cause significant crop losses. Recently, several quantitative resistance loci have been reported and have been shown to confer meaningful levels of nematode resistance in wheat when pyramided together in elite lines (Barloy et al. 2007; Williams et al. 2006).

Wheat has emerged as a model host species for plant-insect interactions. Gene-for-gene interactions between Hessian fly (HF) and its wheat host, are specified by host *R* genes and insect *avr* genes (Harris et al. 2003). At least 32 *R*-gene loci conferring gene-for-gene resistance to HF have been identified in wheat, and in some cases finely mapped pursuant to positional cloning approaches (Kong et al. 2005, 2008; Nsarellah et al. 2003; Sardesai et al. 2005; Zhao et al. 2006). These cases of monogenic or oligogenic resistance have provided entry points into the molecular basis of plant defense against insects. Gene-for-gene interactions are infrequent among other plant-insect interactions, however. Hence, the question remains whether wheat also utilizes indirect defenses, for example, release of volatile chemicals to attract beneficial insectivores. Profiling of volatile chemicals from wheat plants infested by HF shows that these flies do not induce the production of volatiles that is typical of most insect herbivores, which may account for the observed failure of natural enemies to exert population control over HF in wheat crops (Tooker and De Moraes 2007).

Differential display was used to detect cDNAs that are upregulated in response to avirulent HF feeding on plants carrying the *Hf9* resistance gene (Williams et al. 2002). Several *Hessian fly-response* (*Hfr*) genes, *Hfr1, Hfr2 and Hrf3,* have subsequently been shown to encode anti-nutritional lectins, proteins that bind various carbohydrates that affect insect feeding and digestion (Giovanini et al. 2007; Puthoff et al. 2005; Subramanyam et al. 2006). These appear to be relatively general defense response genes, as they can be at least partially induced by virulent and avirulent feeding by several different insects, mechanical wounding, and treatment with methyl jasmonate, salicylic acid and abscisic acid (Giovanini et al. 2007; Puthoff et al. 2005; Subramanyam et al. 2006).

An extensive mRNA profiling experiment involving three wheat cultivars and three HF biotypes was used to compare compatible and incompatible reactions as well as infested versus uninested (Liu et al. 2007a). Notably, five lectin-domain containing genes, including *Hfr1*, were upregulated 5 to 15 fold in incompatible relative to compatible interactions. More importantly though, Liu and colleagues meticulously catalogued results from nearly 200 strongly differentially expressed genes that had putative functions associated with cell wall metabolism, antibiosis, phenylpropanoid biosynthesis, or nutrient metabolism and transport, among others. This list of gene functions is strikingly similar to the list produced by contrasting incompatible and compatible reactions to powdery mildew in barley, demonstrating the commonalities of *R*-gene mediated defense against a wide range of pathogens (Caldo et al. 2004, 2006).

19.6 Pathogen Genomics

Pathogen genomics has become tractable in the light of whole genome sequencing followed by bioinformatic interrogation to uncover genes encoding pathogen effectors, secreted proteins, and transcription factors that facilitate colonization of the host. In addition, transcriptome profiling has prompted a look into gene expression during pathogen attack. Below are updates on six of the major pathogens of the Triticeae.

19.6.1 Fusarium graminearum *(Fusarium Head Blight)*

Fusarium graminearum is the causal agent of head blight (scab) of wheat and barley. In the last decade *Fusarium* head blight (FHB) disease has rapidly re-emerged causing major epidemics in the North America and is becoming a threat globally (Goswami and Kistler 2004). *F. graminearum* (teleomorph *Gibberella zeae*) is a filamentous fungus, belonging to the phylum Ascomycota and order Hypocreales. The *F. graminearum* genome was whole-genome shotgun (WGS) sequenced (strain PH-1) and the resulting assembly totaled 36.45 Mb in 31 scaffolds (www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html).

Genetic mapping of approximately 200 genome-sequence markers anchored 99.8% of the assembly to the genetic map (Gale et al. 2005). Current annotation of the genome contains 13,332 predicted genes. In contrast to other related fungi, *F. graminearum* genome contains few transposons and a low number of paralogoues (Cuomo et al. 2007). The *F. graminearum* genome has greater number of genes for several categories, including transcription factors, hydrolytic enzymes and transmembrane transporters when compared with *Magnaporthe grisea*, *Neurospora crassa* and *Aspergillus niculans*. An extensive set of single-nucleotide polymorphisms (SNPs) were identified by comparing the assembly with a low coverage WGS from a second strain of *F. graminearum* (GZ3639). SNP densities varied across the genome sequence with the highest density in telomeric regions at the ends of the four chromosomes. In addition, three chromosomes had one or two regions with high SNP densities and correlated with regions of highest recombination. These regions of high SNP density have higher frequencies of genes specifically expressed during plant infection and lower frequencies of highly conserved genes.

Güldener and colleagues took advantage of the genome sequence of *F. graminearum* to design a whole-genome (18K) Affymetrix GeneChip (Güldener et al. 2006). To establish a baseline set of gene expression data, *F. graminearum* GeneChips were interrogated with RNA isolated from fungus grown in culture under three nutritional regimes: (a) complete medium (control), (b) minimal medium (Trail et al. 2003) without nitrogen, and (c) minimal medium without carbon, in addition to *in planta* growth in infected barley from the experiment performed by Boddu and associates (Boddu et al. 2006). Interestingly, 7,132 *Fusarium* probe sets were called present during the barley infection time course, even though the fraction of fungal transcripts in the total RNA from infected plants is quite low, notably during the early stages of infection. No enrichment was performed for fungal transcripts and the initial spore density used to inoculate plants was low. However, even at the earliest time point (24 hai), over 100 *Fusarium* sequences were detected.

Recently, WGS assembles for two additional species of *Fusarium*, *F. oxysporum* and *F. verticillioides*, were completed (www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html). Members of the *F. oxysporum* species complex cause vascular wilts of over 100 cultivated plant species including tomato, potato, sugarcane and cowpea, whereas, *F. verticillioides* is the causal agent of kernel and ear rot of maize. The genomes of *F. verticillioides* (41.7 Mb) and *F. oxysporum* (61.36 Mb) are larger than *F. graminearum* and are more complex containing higher levels of repetitive sequences and predicted genes.

19.6.2 Puccinia graminis *(Stem Rust)*

Puccinia graminis, the causal agent of stem rust, as a species has a relatively broad host range including more than 365 species of cereals and grasses

(Leonard and Szabo 2005). Historically, stem rust has been one of the most devastating diseases of wheat and barley. *P. graminis* has been divided into formae specialis based on host range which includes *P. graminis* f. sp. *tritici* (wheat and barley), *P. graminis* f. sp. *avenae* (oat) and *P. graminis* f. sp. *secalis* (rye). Like other rust fungi, *P. graminis* is functionally an obligate biotroph and therefore can only grow on living host tissue. The predominant asexual stage (uredinial) is dikaryotic (n + n) and occurs on its gramineous hosts, where sexual reproduction begins at the resting spore stage (telial) before culminating on the alternate host, barberry (*Berberis* spp.). *P. graminis* is a filamentous fungus, belonging to the phylum Basidomycota and order Pucciniales.

The *P. graminis* f. sp. *tritici* (*Pgt*) genome was WGS sequenced (isolate CDL 75-36-700-3) using DNA from the dikaryotic urediniospores and the resulting assembly totaling 88.6 Mb (including 8% gaps) in 394 supercontigs (www.broad.mit.edu/annotation/genome/puccinia_graminis.2/). This represents the first sequencing of an obligate fungal plant pathogen. Forty-seven percent of the genome is composed of repetitive sequences, with transposons accounting for 12% of the repetitive sequences (C. Cuomo and L. Szabo, unpublished data). Current annotation predicts 20,567 genes covering 36% of the genome. Thirty-one percent of the predicted genes are supported by BLAST or *Pgt* EST data. The *Pgt* secretome is predicted to contain approximately 1,400 proteins, of which 73% are specific to the *Pgt* genome, which is likely of be reduced as additional genomes of rust fungi are sequenced. A partial genetic map has been developed, mapping eight avirulence loci on seven linage groups (Zambino et al. 2000).

19.6.3 Mycosphaerella graminicola *(Septoria Tritici Blotch)*

Mycosphaerella graminicola (anamorph: *Septoria tritici*) is the causal agent of septoria tritici blotch, and is an agronomically important disease in most wheat growing regions worldwide. *M. graminicola* is a haploid, hemibiotrophic fungus with both filamentous and yeast-like growth phases. Early stage of infection is symptomless and intracellular (8–10 days) before a rapid collapse of mesophyll tissue occurs in a susceptible host. *M. graminicola* is an ascomycete in the class Doithdeomycetes and order Capriodiales.

The *M. graminicola* genome was WGS sequenced (isolate IPO323) and the resulting assembly totaling 41.2 Mb in 129 scaffolds with roughly half of the genome contained in 6 scaffolds (http://genome.jgipsf.org/Mycgr1/Mycgr1.home.html). Annotation of this assembly includes a total of 11,396 predicted gene models. The current genetic map is composed of 26 linkage groups (Goodwin et al. 2007). The closely related *M. figiensis*, the causal agent of black leaf streak disease of bananas, has also been recently sequenced (http://genome.jgi-psf.org/Mycfi1/Mycfi1.home.html).

19.6.4 Stagonospora nodorum *(Stagonospora Nodorum Blotch)*

Stagonospora nodorum (teleomorph: *Phaeosphaeria nordorum*) is the causal agent of stagonospora nodorum blotch and is a major pathogen of wheat and barley (Solomon et al. 2006). The most damaging aspect of this disease is the infection of the head, leading to glume blotch. *S. nodorum* is an ascomycete in the class Doithdeomycetes and order Pleosporales.

The *S. nordorum* genome was WGS sequenced (strain SN15) and the resulting assembly totaling 37.1 Mb in 107 scaffolds with more than 50% of the genome contained in the 13 largest (www.broad.mit.edu/annotation/genome/stagonospora_nodorum/Home.html). Approximately 4.5% of the genome is composed of repetitive sequences (Hane et al. 2007). Annotation of the assembled genome predicts at least 10,762 gene models covering 46% percent of the genome. Analysis of ESTs indicates that extracellular proteases, cellulases and xylanases predominate the fungal transcritpome in infected host tissue. The annotated *S. nodorum* genome encodes a large number of secreted proteins in which the majority show no significant homology with genes in current databases.

19.6.5 Blumeria graminis *(Powdery Mildew)*

Blumeria graminis, causal agent powdery mildew of cereals and grasses, has been subdivided into formae specialis based on host range. *B. graminis* f. sp. *hordei* and f. sp. *tritici* exclusively infects barley and wheat, respectively. Like all powdery mildews, *B. graminis* is an obligate biotrophic that, develop specialized feeding structure (haustoria). *Blumeria* epidemics result from a rapid (<5 day) asexual infection cycle, which culminates with the massive production of airborne conidia. The sexual cycle can be observed at the end of the host's growth season when environmental conditions become unfavorable. Outcrossing with compatible strains allows recombination and the generation of new variation on which selection pressure can act. *B. graminis* is an ascomycete in the class Leotiomycetes and order Erysiphales.

The genome of *B. graminis* f. sp. *hordei* (strain DH14) is currently being sequenced by BluGen, a BBSRC (UK)- sponsored international consortium (http://www.blugen.org/). In addition to sequence information on the genome, the website is also a repository for information, publications and images for powdery mildews. As of January 2009, the assembly spans 118 Mb. Initial analysis suggests that the size of the genome is approximately 120 Mb, however there is some uncertainty about the true value of this because of the exceptionally high level of repetitive DNA (60–70%) much of which is not found closely related sequenced ascomycetes (e.g. *Bortytis cinerea* and *Leptospheria maculans*). Preliminary data from high throughput sequencing of other powdery mildews appears to indicate that this might be a common feature amongst

Erysiphales. The BluGen project includes ESTs of conidia, conidia germinated on barley, infected epidermis (including haustoria), non-sporulating and sporulating epiphytic mycelia, and cleistothecia. These ESTs are a useful indication of the spectrum of genes expressed across different developmental stages and are being used to train the *Blumeria*-specific gene finding programs for the initial automated annotation.

Gene expression profiling using a custom *B. graminis* f. sp *hordei* cDNA microarray containing probes covering the *in planta* infection cycle in barley found patterns of coordinate expression among genes in defined metabolic pathways (Both et al. 2005). The authors were also able to assess the metabolic status of the fungus as it infects the host plant during asexual development. Genes encoding several glycolytic enzymes are significantly up-regulated as mature appressoria form. These genes are also seen in the infected epidermis, which contains fungal haustoria. These results correlate with host studies that show an up-regulation of sugar transport and utilization-related genes after infection with *B. graminis hordei* (Caldo, unpublished data; Gjetting et al. 2007) as well as *Arabidopsis* infected with *E. cichoracearum* (Fotopoulos et al. 2003).

19.6.6 Barley Yellow Dwarf Virus *(BYDV)*

Barley yellow dwarf virus (BYDV) and Cereal yellow dwarf (CYDV, formerly a strain of BYDV) are the most widespread and economically important viruses of wheat, barley and oats (D'Arcy and Burnett 1995; McKirdy et al. 2002). They occur wherever these crops are grown. The YDVs comprise a diverse mix of viruses (Miller et al. 2002) making resistance breeding difficult (Gray et al. 1994; Sharma et al. 1995). To begin to understand of the nature of YDVs around the world, the BYDV/CYDV Global Sequencing Project (PI's WA Miller, Iowa State University; SM Gray, Cornell University; J Anderson, Purdue University) commenced in 2004. Unlike projects that sequence multi-megabase genomes of bacterial or fungal pathogens, this small project focused on sequencing multiple, complete ~5,700 nt viral RNA genomes. Given that the viral genome is RNA with no poly(A) tail and that this virus occurs at low levels in plants, obtaining complete sequences was more time consuming and expensive per base than sequencing a DNA genome.

High quality sequences of complete genomes of over 40 YDV isolates from North America and Europe have been determined so far. These sequences revealed: (i) an isolate known as BYDV-RMV is neither BYDV nor CYDV but an entirely new virus; (ii) that a severe isolate, CYDV-RPS known previously to be only in Mexico is widespread throughout western North America and is an entirely different virus from CYDV-RPV; (iii) recombinants between CYDV-RPS and mild CYDV-RPV predominate in the Midwest; (iv) multiple recombination events occur in the coat protein genes suggesting a mechanism to generate virus isolates with new aphid vector specificities; (v) the most common

YDV, BYDV-PAV is remarkably invariant across North America; (vi) phylogenetic relationships of viruses based on structural (coat protein) genes is different from those based on the nonstructural (e.g. polymerase) genes because of the high recombination rate among genomes; (vii) there is a general trend toward less homology among related viruses at the 5′ end of the genome compared to the middle of the genome.

19.7 Synthesis

The interactions between pathogens and plant hosts are a counter-balancing act; as the pathogen attempts to maximize nutrient siphoning and/or colonization, the host aims to restrict nutrient loss while minimizing the cost of defense. Plants initially activate basal defenses to limit penetration and colonization. However, pathogens are often capable of suppressing these innate defenses, requiring the plant to recognize them as threats in order to deploy stronger defenses.

These mechanisms evolved in natural populations, not in crops grown in monoculture. The defeat of recognition mechanisms by crop pathogens is now expected, requiring breeders to pyramid several *R* genes to ensure lasting protection against any single pathogen. Unfortunately, the threat of multiple pathogens in many growing regions makes it impractical to maintain optimal defense against each. A promising solution lies in the deployment of improved basal defense, which acts against a broad spectrum of pathogens and is not based on specific recognition, increasing its durability. But first, we must discover the molecular basis for more of these mechanisms across a wider spectrum of pathogens.

The state of the science for host-pathogen interactions in the Triticeae is encouraging. Well-developed experimental germplasm for targeted experiments in many emerging systems and the availability of large datasets from previous efforts present excellent opportunities for future analysis. The emergence of genetical genomic methods that attach cause to nucleic acid sequences, rather than simply to regions of the genome, is particularly encouraging. Likewise, advancements in high-throughput transient and virus-induced manipulations of gene expression make possible a wide variety of experiments that will be required to decipher intricacies of basal resistance, nonhost resistance, as well as race-specific resistance. These resources will promote the understanding of the complex nature of their interactions among Triticeae hosts and their pathogens, which will have long-term value for crop improvement.

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